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Temnocephala pignalberiae Dioni, 1967 (Platyhelminthes, Temnocephalida) from two allopatric populations of *Dilocarcinus pagei* Stimpson, 1861 (Crustacea, Decapoda) — first record for Brazil

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Abstract

The presence of adult specimens and eggs of *Temnocephala pignalberiae* Dioni, 1967 in *Dilocarcinus pagei* Stimpson, 1861, at Poconé, Brazilian 'Pantanal', State of Mato Grosso, together with abundant material also collected from *D. pagei* at Bebedouro, State of São Paulo, are reported. These two records allowed the extension of the known geographical distribution of *T. pignalberiae* from Argentina to Central Brazil, and the comparison of the temnocephalan infrapopulations from two allopatric crab populations, living approximately 1,300 km apart from each other. The entire sample from Poconé and part of the sample from Bebedouro were fixed according to our protocols. Juvenile and adult temnocephalans were whitish to light pink and presented the typical red-eye pigmentation. The worms were living in the branchial chambers, where they deposited their eggs, over the tissue covering the inner upper part of the carapace. These eggs were thin-shelled and the most delicate, so far recorded, for any temnocephalan species our group has studied. The most distinctive features of the temnocephalan specimens from the two localities representing different watersheds were the: 1. cirrus with unarmed introvert; 2. prostatic bulb thick-walled; 3. body ellipsoid, with small and more separate tentacles; 4. syncytial plates elongated and with similar shape; and 5. excretory pores in the same position inside the each syncytial plate. Differing minimally by the: 1. contrasting shaft bases – specimens from Poconé, with rims of the shaft base directed inward, while specimens from Bebedouro, with straight and more open shaft base rims, similar to the shaft bases drawn based on specimens found in Argentina; and 2. ratio between cirrus length and prostatic bulb length, resulting in longer prostatic bulbs in the specimens from Bebedouro. Statistical analysis of measurements taken from flattened adult specimens from the infrapopulations of the two localities showed only minimal differences in the length and width of some organs. This is expected to occur when the same species is collected from populations of the same host species living far from each other and representing separate watersheds.

Key words: Geographical distribution, Neotropic Region, State of Mato Grosso, State of São Paulo, taxonomy, temnocephalans

Introduction

Crabs of the genus *Dilocarcinus* H. Milne-Edwards, 1853 (Dilocarcinini Pretzman, 1978) are present in all South American lowlands, from Guyana to Argentina; those of the genus *Sylviocarcinus* H. Milne-Edwards, 1853 (Valdiviinae Pretzman, 1978) are present in all larger river drainage systems of South America, usually associated with floating meadows, from Venezuela to Argentina (Magalhães, 2003; Magalhães, 2005; Magalhães & Turkay, 1996). These crabs were first reported to have temnocephalans as ectocommensals by Pignalberi (1962), in Argentina, the southernmost area of their known distribution. Dioni (1967) described the species *Temnocephala pignalberiae* from *Trichodactylus orbicularis* Meuschen, 1781 (= *Dilocarcinus pagei* Stimpson, 1861), collected from Río Salado, and Laguna de Los Espejos, Santo Tomé, Province of Santa Fe,

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Argentina, also from *Trichodactylus pictus* H. Milne-Edwards, 1853 (= *Sylviocarcinus australis* Magalhães & Türkay 1996) collected from Laguna Guadalupe, Santo Tomé, Province of Santa Fe, Argentina and specimens of an unidentified species of *Trichodactylus* collected from Madrejón "Don Felipe", Province of Santa Fe, Argentina and the outskirts of Paraná, Entre Ríos, Argentina. Later, in 1992, the species was collected from live crabs, *D. pagei* and *Sylviocarcinus pictus* (Milne-Edwards, 1853), from Laguna Guadalupe in the same province (Damborenea, 1992). At that time the known geographical distribution of *T. pignalberiae* was restricted to Argentina.

In Brazil, Rego (1982) recorded *D. pagei* and *Valdivia serrata* White, 1847, from two localities in the State of Mato Grosso, as being infested by temnocephalans, which the author identified only as *Temnocephala* sp. The present work records the presence of *T. pignalberiae* in Brazil, but also records two allopatric host populations infested by the species, and for the first time documents photographically the unusual egg deposition site (already mentioned by Damborenea (1992)), their shape and delicate shell, the body shape when fixed with hot formalin, with and without slight pressure, and the shape of the paired, dorsolateral, post-tentacular 'excretory' syncytial plates (DLSPs) as revealed by scanning electron microscopy (SEM).

Material and methods

Crabs were collected in two separate areas, in the State of Mato Grosso and in the State of São Paulo. One of us (JFRA), examined seven specimens of *D. pagei* collected from shallow pools, usually inundated during the flooding season in the Brazilian Pantanal (from December to April), located on the property of Fazenda Ypiranga (16º20'14.26"S, 56º38'58.22"W), 10 km south of Poconé, MT and 110 km from Cuiabá, MT, the capital of the State of Mato Grosso (from now on referred, generically, as Poconé). In the city of Bebedouro, (20º56'58.44"S, 48º28'43.90"W), State of São Paulo, two of us (MF and RJI) collected 100 crabs identified as *D. pagei* (56 males and 44 females, ranging from 28 to 40 mm across the width of the carapace), from November 2004 to August 2005, from the first dam in the Arroio Candinho, property of the Ecological Station, city of Bebedouro. Crabs were collected manually or with a dip nets and taken still alive to the Laboratório de Biologia das Faculdades Integradas (Fafibe). Measurements appearing in Table 1 are in micrometers (µm) unless otherwise indicated and were taken from specimens killed under slight cover slip pressure and mounted in Canada balsam; ranges are followed (between parentheses) by the mean, the standard deviation values, and the number of specimens measured for a given character (when different than 8 for Poconé and 12 for Bebedouro). Measurements of cirrus length were taken from extracted cirri mounted in Faure´s mounting medium (F), according to Cannon 1993, Cannon & Sewell 1995, and Sewell & Cannon 1998. The terminology used to describe the male reproductive structures follows Amato *et al.* (2007) and Seixas *et al*. (2010). The whole mounts of adult and juvenile voucher specimens, as well as slides containing individual cirri and sometimes some feminine organs mounted in Faure´s mounting medium were deposited in the following scientific collections: 1. "Coleção Helmintológica do Instituto Oswaldo Cruz" (CHIOC), Rio de Janeiro, RJ, Brazil; 2. "Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia" (INPA), Manaus, AM, Brazil; 3. "Colección de Invertebrados, División Zoologia Invertebrados, Museo de La Plata" (MLP), La Plata, Argentina; and 4. "Coleção Helmintológica do Laboratório de Helmintologia" (SBA - acronym meaning Suzana Bencke Amato, for the invertebrate hosts), Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS. One host specimen from Bebedouro, SP, was deposited in the "Coleção de Crustáceos, Departamento de Zoologia, UFRGS", while another host was kept in the invertebrate host collection of the Laboratório de Helmintologia.

The temnocephalans from *D. pagei*, were studied through a series of techniques fully described by Amato *et al.* (2007) and Seixas *et al.* (2010), focusing especially on the: 1. areas of egg deposition in the host; 2. shape, position of the filament, and thickness (degree of tanning) of the egg shell; 3. cirrus structure and some feminine organs as revealed by F preparations, phase contrast microscopy, and Nomarski´s differential interference contrast (DIC); and 4. shape of the DLSPs, as revealed by SEM and relative position of the excretory pore. The present paper establishes a comparison of the temnocephalans from allopatric crab populations collected from localities in separate watersheds more than 1,300 km apart.

Results

***Temnocephala pignalberiae* Dioni, 1967**
(Figs. 3–37)

Study based on 57 specimens collected: 26 specimens stained in hematoxylin mounted *in toto*: 8 from Poconé and 18 from Bebedouro; 6 specimens stained in aceto-carmine/fast green mounted *in toto*: 5 from Poconé and 1 from Bebedouro; 22 extracted cirri mounted in Faure´s medium: 5 from Poconé and 17 from Bebedouro; and 3 slides with eggs mounted in Canada balsam: 2 from Poconé and 1 from Bebedouro.

Table 1 presents morphometric data comparing specimens measured from Poconé and Bebedouro.

Taxonomic summary. **Type host and type locality:** Dioni (1967) did not indicate the type host and the type locality for this species: "*Trichodactylus orbicularis* Meuschen, Río Salado, Santo Tomé, Argentina; *Trichodactylus pictus*, Madrejón Don Felipe y Laguna Guadalupe, Santa Fe, Argentina; *Trichodactylus* sp., Madrejón Don Felipe, Santa Fe; *Trichodactylus* sp., alrededores de Paraná, Entre Ríos; *T. pictus* y *T. orbicularis*, Madrejón Don Felipe, Santa Fe; *T. pictus*, Laguna Guadalupe, Santa Fe".

Other hosts and localities: Argentina: "*Dilocarcinus pagei* Stimpson, 1861, Laguna Guadalupe (Piedras Blancas), Santa Fe, Argentina (Damborenea, 1992)". Brazil (present work): *Dilocarcinus pagei* in Poconé, State of Mato Grosso and in Bebedouro, State of São Paulo.

Site of infestation: adults found inside the branchial chambers; eggs deposited over the tissue covering the inner upper part of the carapace.

Prevalence: 100%, in both localities, Poconé, MT and Bebedouro, SP.

Specimens deposited: CHIOC Nº 37307 (SBA-2801-2-2, eggs), CHIOC Nº 37308 (SBA-2807-1-3, specimen *in toto*), CHIOC Nº 37309 (SBA-2799-1-5, cirrus in F), CHIOC Nº 37310 a-b (SBA-3076-1-1 (a), specimen *in toto*; SBA-3076-1-6 (b), specimen *in toto*), CHIOC Nº 37311 (SBA-2944-1-1, cirrus in F); INPA Nº 525 (SBA-3076-1-5: specimen *in toto*), INPA Nº 526 (SBA-2945-1-4: cirrus in F), INPA Nº 527 (SBA-2799-1-3: specimen *in toto*), INPA Nº 528 (SBA-2800-1-2: cirrus in F); MLP Nº 6091 (SBA-2807-1-1: specimen *in toto*), MLP Nº 6092 (SBA-2799-1-4, cirrus in F), MLP Nº 6093 (SBA-2802-2-1, eggs), MLP Nº 6094 (SBA-2946-1-4: specimen *in toto*), MLP Nº 6095 (SBA-2945-1-3, cirrus in F).

Specimens examined: "*Temnocephala pignalberiae*, MLP Nº 3126, camaras branquiales, *Dilocarcinus* (*D.*) *pagei*, Laguna Guadalupe, Santa Fe, Argentina, col. 2 Febrero 1987"; "*Temnocephala pignalberiae*, MLP Nº 3127, camaras branquiales, *Sylviocarcinus pictus*, Laguna Guadalupe, Santa Fe, Argentina. Col. 2 Febrero 1987"; "*Temnocephala pignalberiae*, MLP Nº 3127, camaras branquiales, *Sylviocarcinus pictus*, Laguna Guadalupe (Piedras Blancas), Santa Fe, Argentina. Col. 1 Febrero 1987"; and "*Temnocephala pignalberiae*, MLP" (slide without collection number, host name, date of collection, and collector indication).

Host specimens deposited: "Coleção de Crustáceos UFRGS Nº 4565 – SBA 3184 – *Dilocarcinus pagei,* Bebedouro Col. R. J. Ilário"; "Coleção de Hospedeiros Laboratório de Helmintologia UFRGS – SBA 3185 – *Dilocarcinus pagei,* Bebedouro. Col. R. J. Ilário."

Comments. Dioni (1967) did not indicate having elected and deposited a holotype and paratypes of *T. pignalberiae*; consequently, he also did not indicate which of the two species of crabs, *D. pagei* or *S. australis*, would be the type host, nor did he indicate which of the five collection localities would be the type locality for the species. Later, Damborenea (1992) and Damborenea & Cannon (2001) collected again *T. pignalberiae* from the same hosts and from the same localities in Argentina.

Temnocephala pignalberiae is the species with the most perfect elliptical body shape (when properly killed/fixed by formalin 10% formalin 90ºC — compare Figs. 8–11 with Figs. 12 and 13) and the smallest tentacles, among the species so far studied. The crabs infested with *T. pignalberiae* did not have temnocephalan eggs deposited on the external areas of the carapace, abdomen or pereiopods (Figs. 1 and 2). The eggs laid by *T. pignalberiae* are the most delicate which we have found in any of the species studied. Additionally, these eggs being laid over the tissue which covers the inner upper surface of the carapace were collected by 'peeling off' this tissue (Figs. 4 and 5). These eggs have a long, subterminal filament (Fig. 6). In the present paper, specimens of *D. pagei,* from Poconé and Bebedouro, were infested exclusively by *T. pignalberiae*, which allowed us to be certain about the morphology and the correct place for the deposition of eggs by this species.

TABLE 1. Comparison between the specimens of *Temnocephala pignalberiae* from *Dilocarcinus pagei* of two allopatric populations in Poconé, State of Mato Grosso and Bebedouro, State of São Paulo, respectively.

Characters	Present work	Present work	Student´s *t* test	
	Poconé, Mato Grosso (n = 8)	Bebedouro, São Paulo (n = 12)	t_{calc}	*p*
Total body length without tentacles	1.92–3.22 mm (2.59 mm ± 488)	1.68–3.90 mm (2.49 mm ± 679)	0.3609	0.7223
Total body width	1.32–2.33 mm (1.86 mm ± 375)	1.09–2.38 mm (1.68 mm ± 443)	0.9334	0.3680
Adhesive disk length	380–889 (694 ± 187)	410–900 (597 ± 166; 11)	—	—
Adhesive disk width	460–830 (687 ± 151)	400–931 (603 ± 176; 11)	—	—
Adhesive disk peduncle length	230–490 (373 ± 81)	150–594 (359 ± 117; 11)	—	—
Pharynx length	350–553 (455 ± 70)	230–740 (499 ± 160)	—	—
Pharynx width	350–612 (487 ± 94)	300–713 (441 ± 122)	—	—
GLANDS				
Rhabditogenic glands diameter	40–100 (71 ± 21)	30–95 (62 ± 16)	1.1060	0.2832
Haswell glands largest cell diameter	63–113 (86 ± 17; 7)	50–125 (78 ± 26; 10)	—	—
Disk glands diameter	40–63 (50 ± 8)	30–60 (40 ± 11; 7)	—	—
Large adhesive disk glands length	63–113 (95 ± 24; 4)	48–138 (76 ± 31; 7)	—	—
FEMALE REPRODUCTIVE SYSTEM				
Ovary length	83–148 (114 ± 25)	65–145 (107 ± 28)	0.5917	0.5614
Ovary width	68–128 (94 ± 19)	85–130 (110 ± 15)	-2.1805	0.0427*
Vagina length	65–108 (92 ± 17; 6)	78–138 (107 ± 17; 8)	-1.7008	0.1146
Vagina width	70–88 (80 ± 6; 6)	68–100 (83 ± 11; 10)	-0.6985	0.4957
Vaginal sphincter total diameter	53–60 (56 ± 3; 4)	63–80 (71 ± 12; 2)	-2.5512	2.65
Vaginal sphincter anterior diameter	25–30 (27 ± 2; 4)	25–30 (28 ± 3; 2)	-0.2649	0.8042
Vaginal sphincter posterior diameter	25–28 (26 ± 1; 4)	30–38 (34 ± 5; 2)	-2.9542	0.0418*
Seminal receptacle diameter	15–26 (23 ± 7; 2)	15–30 (21 ± 8; 3)	0.2774	0.7953
Vesicula resorbens length	100–150 (127± 16)	80–228 (147 ± 47; 10)	—	—
Vesicula resorbens width	143–240 (192 ± 33)	118–278 (197 ± 46; 10)	—	—
Vesicula resorbens wall thickness	5–13 (9 ± 2)	5–18 (10 ± 4; 10)	—	—
Egg length	370–430 (400 ± 17; 13)	—	—	—
Egg width	180–220 (208 ± 12; 7)	—	—	—
Peduncle length	10–70 (30 ± 19; 7)	—	—	—

continued next page

TABLE 1. (continued)

Characters	Present work	Present work	Student´s *t* test	
	Poconé, Mato Grosso (n = 8)	Bebedouro, São Paulo (n = 12)	t_{calc}	*p*
MALE REPRODUCTIVE SYSTEM				
Right anterior testis length	170–290 (227 ± 39; 7)	138–310 (207 ± 50)	—	—
Right anterior testis width	190–350 (238 ± 50)	130–250 (175 ± 38)	—	—
Right posterior testis length	190–350 (269 ± 50)	163–370 (246 ± 59)	—	—
Right posterior testis width	180–360 (258 ± 58)	163–350 (224 ± 59)	—	—
Left anterior testis length	120–240 (203 ± 43; 6)	138–390 (218 ± 72)	—	—
Left anterior testis width	200–250 (217 ± 23; 6)	130–260 (187 ± 44)	—	—
Left posterior testis length	190–320 (249 ± 49)	163–470 (260 ± 79)	—	—
Left posterior testis width	160–430 (288 ± 80)	163–280 (220 ± 41)	—	—
Seminal vesicle length	156–245 (198 ± 30)	110–333 (183 ± 67; 10)	0.6425	0.5326
Seminal vesicle width	60–90 (72 ± 1)	53–90 (77 ± 12; 11)	-0.8198	0.4236
Seminal vesicle wall thickness	8–15 (10 ± 3)	10–23 (14 ± 3; 11)	—	—
Prostatic bulb length	125–178 (155 ± 19)	120–200 (160 ± 26)	-0.4512	0.6572
Prostatic bulb width	75–100 (90 ± 9)	75–108 (92 ± 11)	-2.5185*	0.0269*
Prostatic bulb wall thickness	18–28 (21 ± 3)	13–25 (19 ± 4)	—	—
Cirrus length	100–113 (105 ± 5; 14)	100–113 (105 ± 4; 14)	—	—
Shaft length	78–93 (85 ± 5; 14)	78–93 (85 ± 5; 14)	—	—
Shaft width at base	28–40 (34 ± 4; 14)	28–40 (34 ± 4; 14)	—	—
Introvert length	15–23 (18 ± 2; 14)	15–23 (18 ± 2; 14)	—	—
Introvert width at base	13 (13 ± 0; 14)	13 (13 ± 0; 14)	—	—
Introvert maximum width	10–15 (15 ± 2; 14)	10–15 (13 ± 2; 14)	—	—

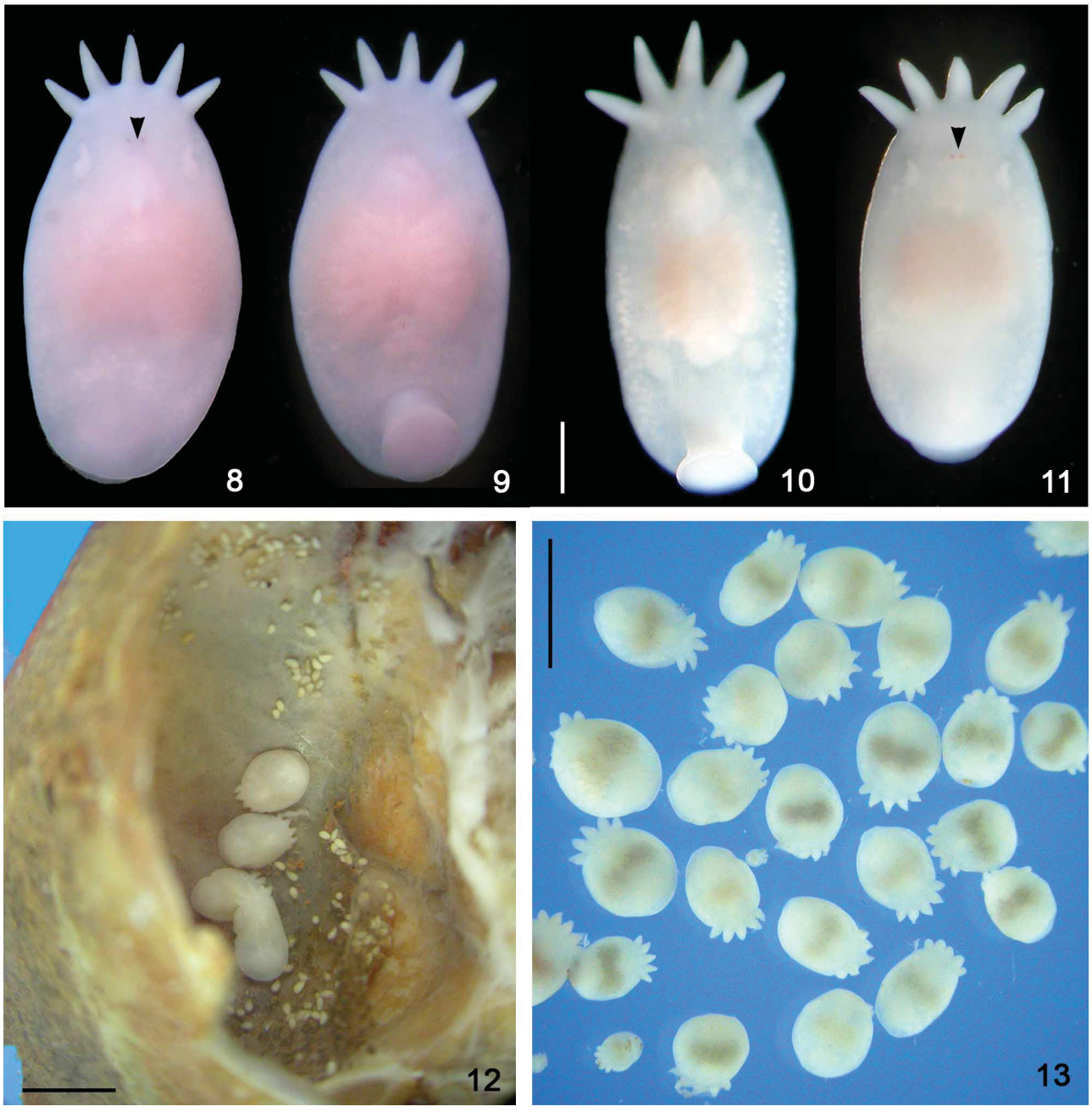
* = significant values of *p*.

FIGURES 1–2. *Dilocarcinus pagei*, showing the absence of eggs of *T. pignalberiae* on the external dorsal and ventral surfaces, including the pereiopods. 1. adult female specimen in dorsal view. Scale bar = 1.5 cm. 2. adult female specimen in ventral view. Scale bar = 1.5 cm. **FIGURES 3–7**. *Temnocephala pignalberiae*. 3. live eggs deposited on the tissue (asterisks) covering the inner upper surface of the carapace. Scale bar = 500 µm. 4. fixed eggs cemented to the tissue covering the inner upper surface of the carapace. Scale bar = 1 mm. 5. fixed eggs cemented to the tissue covering the inner upper surface of the carapace, in higher magnification (two asterisks). Scale bar = 500 µm. 6. unhatched egg showing the filament (black head arrow). Scale bar = 250 µm. 7. adult, killed in hot formalin showing the correct shape of the body, stained in aceto-carmine/fast green. Scale bar = 1 mm.

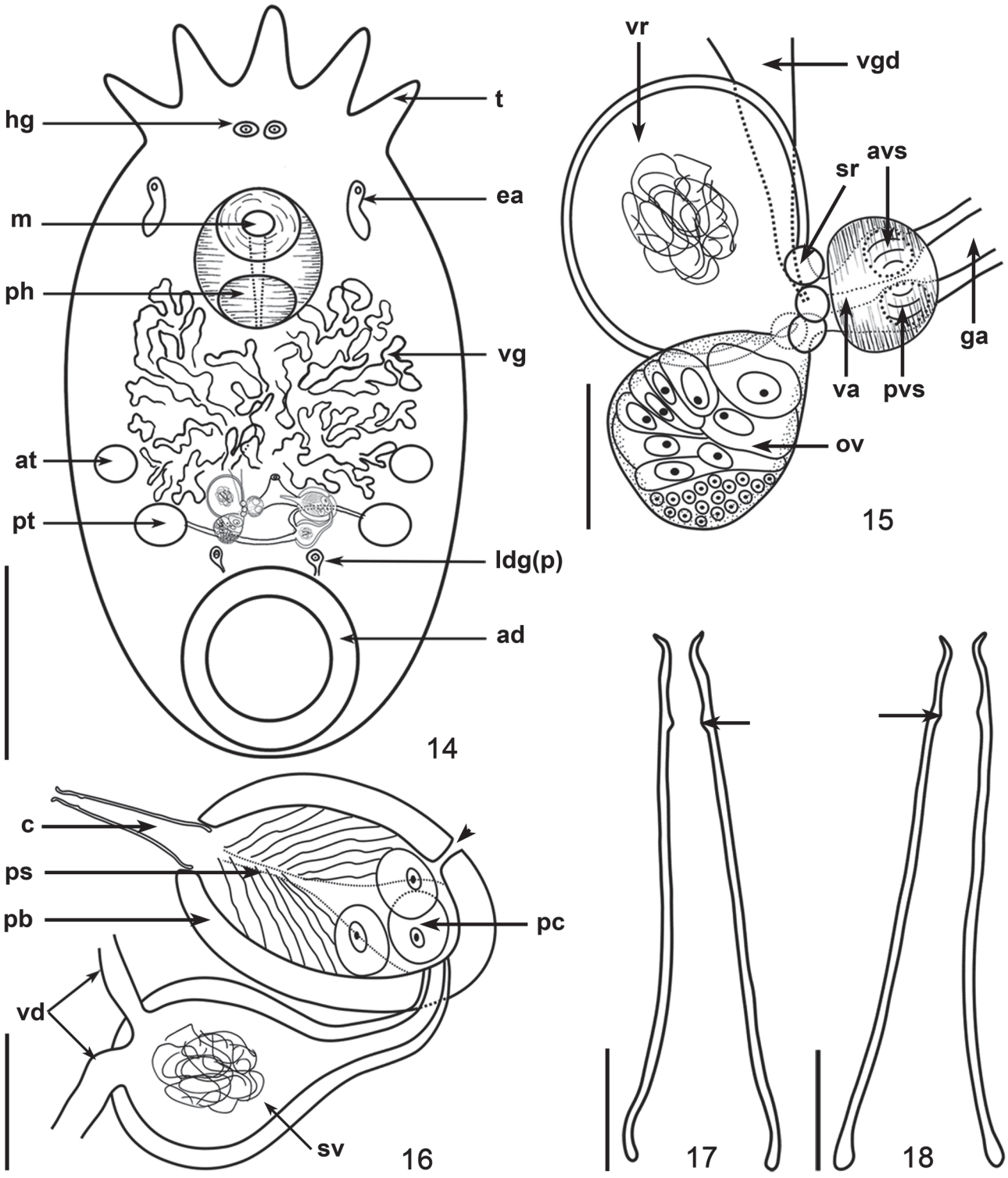
Specimens of *T. pignalberiae* from these two allopatric populations of *D. pagei* showed many similarities when compared: above all, the shape of the cirrus, which is the only unarmed (without spines in the introvert) cirrus found in species of *Temnocephala*. The cirri in specimens from Poconé are proportionally longer, in relation to the length of the prostatic bulbs. The latter have thicker walls and are less elongate than those found

in specimens from Bebedouro. In specimens from Poconé the proximal end of the shaft tends to have the rims curved inward, when compared to the rims of the shaft of cirri in the specimens from Bebedouro. The prostatic bulb is more elongated (pyriform) than elliptical, and the rims of the proximal extremity of the shaft are not curved inward (Figs. 17 and 18; 24 and 29).

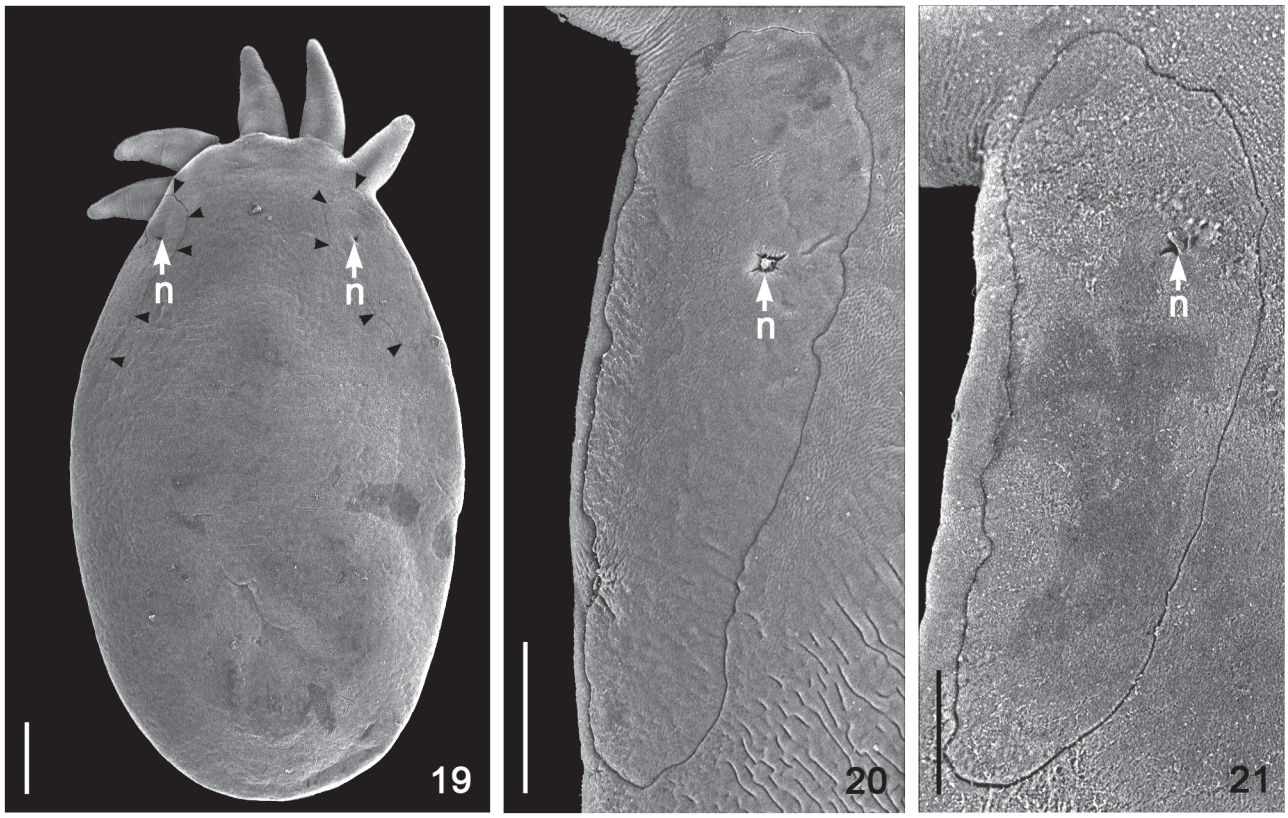
Temnocephala pignalberiae has only one vaginal sphincter, which appears to be double when observed in whole mounts, because the distal portion of the vagina has very thick walls, and can be clearly observed when DIC illumination is applied to dissected reproductive parts mounted in Faure´s medium (Fig. 32) or in specimens stained with aceto-carmine/fast green (Fig. 37).



FIGURES 8–13. *Temnocephala pignalberiae.* 8–9. specimens from Poconé fixed in hot formalin showing the typical body shape and the red-eyes (black head arrow). 10–11. specimens from Bebedouro fixed in hot formalin showing the typical body shape and the red-eyes (black head arrow). Same scale bar to Figs. 8-11 = 500 µm. 12. carapace of crab killed with cold ethanol showing the specimens and many eggs cemented to the tissue which covers the inner upper surface of the carapace. Scale bar = 2 mm. 13. specimens killed with cold ethanol, together with the host, showing the altered body shape. Scale bar = 2 mm.



FIGURES 14–18. *Temnocephala pignalberiae.* 14. incomplete diagram of an adult specimen, ventral view, showing: adhesive disk (ad), anterior testis (at), excretory ampullae (ea), dorsal vitelline glands (vg), Haswell glands (hg), large disk glands (paranephrocytes?) (ldg (p)), mouth (m), posterior testis (pt), pharynx (ph), and tentacles (t). Scale bar = 1 mm. 15. female reproductive organs: anterior portion of the vaginal sphincter (avs), genital atrium (ga), ovary (ov), posterior portion of the vaginal sphincter (pvs), vagina (va), vesicula resorbens (vr), vitelline glands duct (vgd), seminal receptacles (sr). Scale bar = 50 µm. 16. male reproductive system: cirrus (c), opening in the prostatic bulb wall (black head arrow), prostatic bulb (pb), prostatic cells (pc), prostatic secretion (ps), seminal vesicle (sv), and vasa deferentia (vd), Scale bar = 50 µm. 17. cirrus of specimens from Poconé, proximal limit of the introvert (arrow), proximal shaft base curving in. Scale bar = 25 µm. 18. cirrus of specimens from Bebedouro, proximal limit of the introvert (arrow), proximal shaft base not curving in. Scale bar = 25 µm.



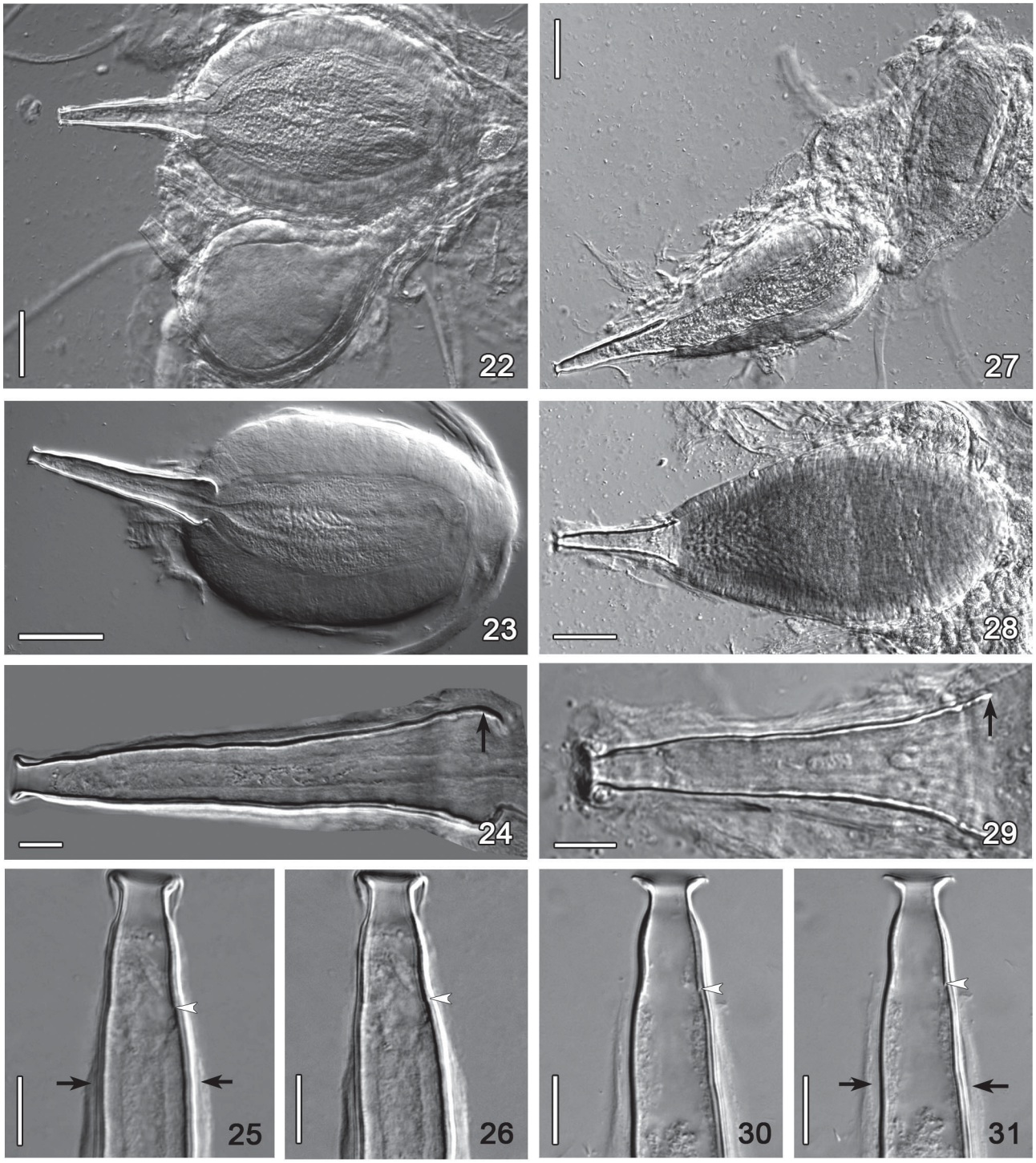
FIGURES 19–21. *Temnocephala pignalberiae* seen with SEM. 19. entire specimen from Poconé, showing the dorsolateral 'excretory' syncytial plates (black head arrows) and position of excretory pores (n – white arrows). Scale bar = 200 µm. 20. left DLSP, seen in a specimen from Poconé, (n – white arrow). Scale bar = 100 µm. 21. left DLSP, seen in a specimen from Bebedouro, (n – white arrow). Scale bar = 100 µm.

Other similarities observed between the specimens from *D. pagei*, of Poconé and Bebedouro were the shape of the DLSPs, as revealed by SEM (Figs. 20–21); the presence of two large disk glands (paranephrocytes?); and the dorsal position of the vitellarium, which is well superposed to the intestinal sac and has the terminal portions of the branches turning toward its ventral side (Figs. 7 and 34).

Discussion

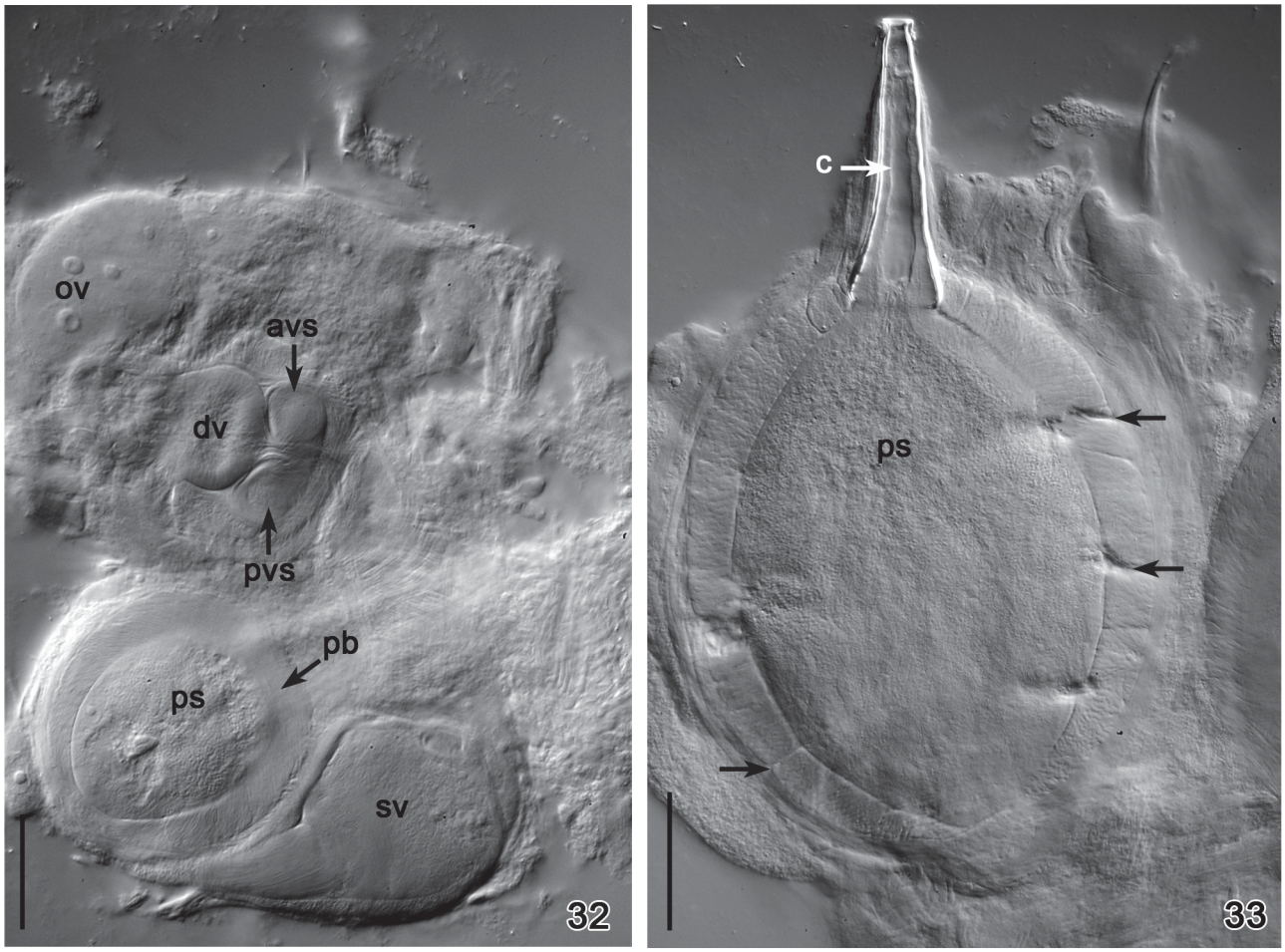
Dioni (1967) studied specimens of *T. pignalberiae* collected by Clarice Pignalberi and deposited in jars at the Museo de Ciencias Naturales y Antropología de Paraná, Province of Entre Ríos. Other crabs studied by Dioni had concurrent infestations of *T. pignalberiae* with *Temnocephala microdactyla* Monticelli, 1903, at Laguna de Guadalupe, Province of Santa Fe. In the same paper, Dioni recorded the presence of an undescribed species, which he described as *Temnocephala santafesina* Dioni, 1967, found also in *T. orbicularis* (= *D. pagei*). This species was living in concurrent infestations with *T. pignalberiae*, at Madrejón Don Felipe, Province of Santa Fe. The author also collected *T. pignalberiae* in single infestations at Río Salado, Santo Tomé, Province of Santa Fe.

The original drawing of an adult specimen made at the time of the species description (Dioni, 1967, Fig. 2a, pg. 351) is somewhat different than the adults collected in both localities during the present work, which are longer and elliptical, with small tentacles. The shape of the body may change accordingly with the method of fixation. When the crab host is placed directly in ethanol the helminth body contracts and appears rounded, with the general shape of any other species (Fig. 13), while the typical body shape, truly elliptical for *T. pignalberiae*, can only be observed when the helminths are killed with 10% formalin 90ºC (Figs. 8–11), maintaining for some time the red pigmentation of the eyes, which is usually diluted by the ethanol.



FIGURES 22–26. *Temnocephala pignalberiae*, photomicrographs of seminal vesicle, prostatic bulb, and cirrus, dissected from specimens from Poconé, mounted in Faure´s mounting medium, observed with Nomarski´s DIC microscopy. 22. seminal vesicle, prostatic bulb, and cirrus. Scale bar = 50 µm. 23. prostatic bulb and cirrus. Scale bar = 50 µm. 24. cirrus, showing the proximal end of the shaft curving in (black arrow). Scale bar = 10 µm. 25–26. cirrus introvert observed in two different focusing planes, indicating the proximal limit of the spineless introvert (white head arrow) and the retractor muscles (black arrows). Scale bar = 10 µm.

FIGURES 27–31. *Temnocephala pignalberiae,* photomicrographs of seminal vesicle, prostatic bulb, and cirrus, dissected from specimens from Bebedouro, mounted in Faure´s mounting medium, observed with Nomarski´s DIC microscopy. 27. seminal vesicle, prostatic bulb, and cirrus. Scale bar = 50 µm. 28. prostatic bulb and cirrus. Scale bar = 50 µm. 29. cirrus, showing the proximal end of the shaft not curving in. Scale bar = 10 µm. 30–31. cirrus introvert observed in two different focusing planes, indicating the proximal limit of the spineless introvert (white head arrows) and the retractor muscles (black arrows). Scale bar = 10 µm.

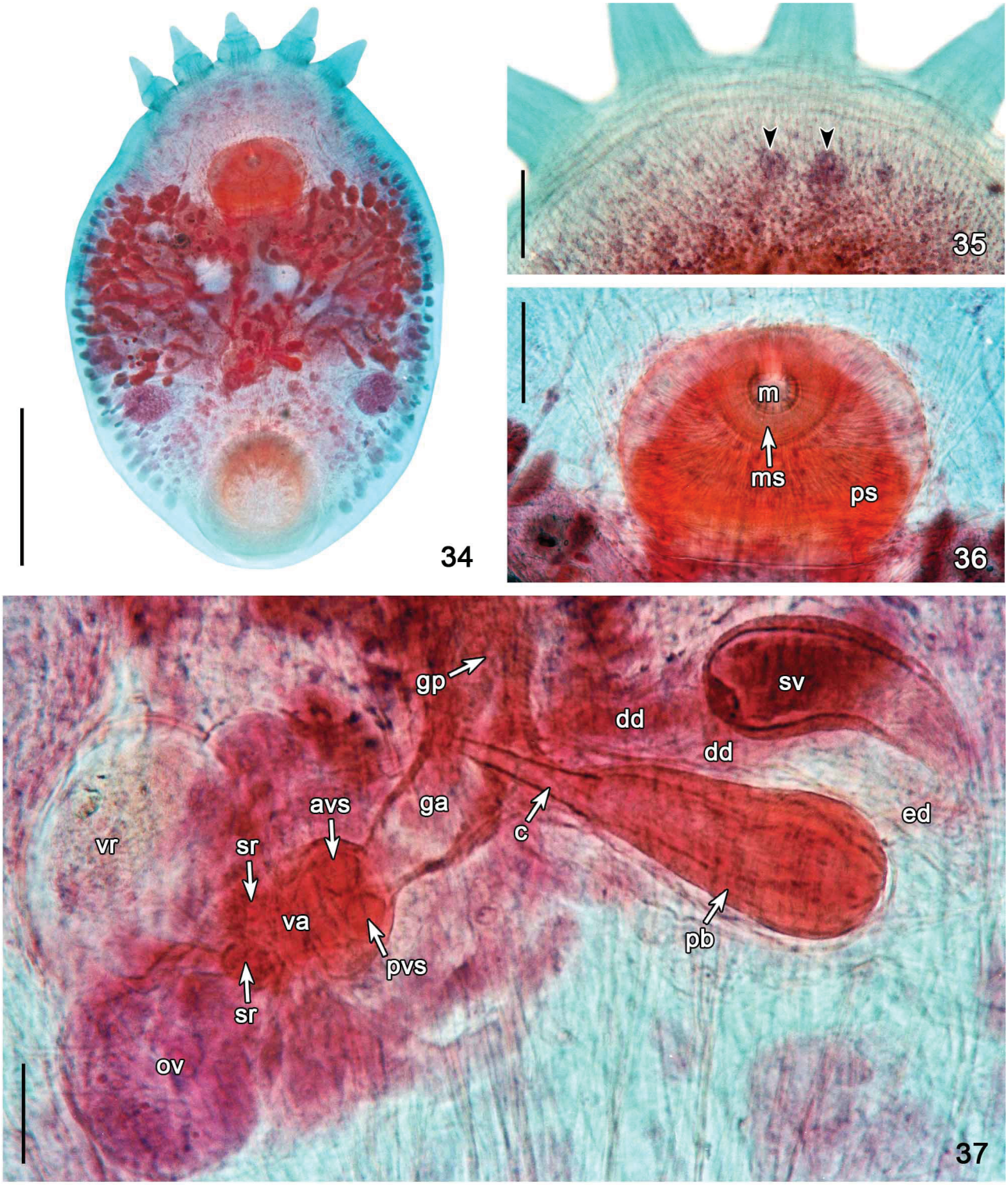


FIGURES 32–33. *Temnocephala pignalberiae.* 32. partial view of the reproductive system, observed with Nomarski´s DIC microscopy: male organs – seminal vesicle (sv), prostatic bulb (pb), and prostatic secretion (ps); female organs – ovary (ov), distal vagina (dv), vaginal sphincter with the anterior vaginal portion (avs), and the posterior vaginal portion (pvs). Scale bar = 50 µm. 33. enlarged prostatic bulb showing the wall openings (black arrows), the prostatic secretion (ps), and cirrus (c), both from a specimen from Poconé. Scale bar = 50 µm.

Dioni (1967) did not address the aspect of the egg deposition sites, although Damborenea (1992) has mentioned that the eggs of *T. pignalberiae* were deposited in the "techo, piso y los tres primeros pares de branquias" *i. e.* "ceiling, floor and the first three pairs of gills", but did not document her observation. If, eventually, *D. pagei* crabs are found with temnocephalan eggs deposited on the external surfaces of the body, the eggs certainly will belong to a different species than *T. pignalberiae*.

The additional drawings presented by Dioni (1967, Figs. 2b and 2c, pg. 351) to illustrate the cirrus and the prostatic bulb, together with the cirrus, agree in general shape and proportion with what was observed in the specimens from Bebedouro. The measurements made by this author, based on 31 specimens collected from two crabs, are in average (2.35 mm long, 1.39 mm wide), much smaller than those we have found for specimens collected in Bebedouro and Poconé (Table 1).

Dioni (1967) did not mention the presence of a vaginal sphincter in *T. pignalberiae* while Damborenea (1992) illustrated one strong vaginal sphincter. The present work confirms this observation made by the latter author, which can be better demonstrated when DIC illumination is applied to dissected female reproductive organs mounted in Faure´s medium (Fig. 32) than when specimens are stained with aceto-carmine/fast green (Fig. 37). Because the proximal portion of the vagina has very thick walls it may appear to some as a second sphincter (Fig. 32).



FIGURES 34–37. *Temnocephala pignalberiae.* 34. adult specimen pressed between slide and cover slip, showing that too much pressure may be good to allow all characters to be observed in the same focusing plane, but alters the body shape. Scale bar = 1 mm. 35. Haswell glands (black head arrows). Scale bar = 200 µm. 36. mouth (m), mouth sphincter (ms), and pharyngeal sphincter (ps). Scale bar = 200 µm. 37. male and female reproductive system: anterior portion of the vaginal sphincter (avs), cirrus (c), deferent duct (dd), ejaculatory duct (ed), genital pore (gp), genital atrium (ga), ovary (ov), posterior portion of the vaginal sphincter (pvs), prostatic bulb (pb), seminal receptacles (sr), seminal vesicle (sv), vagina (va), and vesicula resorbens (vr). Scale bar = 100 µm.

Even though some authors (Sewell *et al.* 2006) did not consider the value of documenting the shape of the DLSPs and the relative position of the excretory pore, both clearly evidenced by SEM and/or by silver nitrate, in the present paper it was possible to demonstrate that these syncytial plates are quite similar (Figs. 20-21), as well as the position of the excretory pore inside each syncytial plate, thus contributing to the specific determination of the temnocephalans from both localities. The dorsal position of the vitellarium in relation to the intestinal sac and the presence of two large disk gland cells (ldg (p) - Fig. 14)) (paranephrocytes?) immediately behind the reproductive organs were not described by Dioni (1967) or Damborenea (1992).

Damborenea (1992), in her study of the temnocephalan species from crustaceans and mollusks of Argentina, collected *T. pignalberiae* infesting *S. pictus* and *D. pagei* at Laguna Guadalupe (Piedras Blancas), Province of Santa Fe, and found measurements of adult temnocephalans to be slightly larger than those of Dioni. In both accounts, these authors reported length measurements of adult specimens, but did not indicate if the total length measured included the tentacles or not. In the present work the total length of the body measured did not include tentacles. Damborenea & Cannon (2001) also did not include measurements of the specimens of *T. pignalberiae* collected from crabs at Laguna Guadalupe.

In Brazil, Rego (1982) examined specimens of *D. pagei* and *V. serrata* from Rio Alegre and Rio Guaporé, Vila Bela, approximately (15º00'48.12"S, 59º57'56.37"W), State of Mato Grosso and found temnocephalans, but identified them only as *Temnocephala* sp.

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References

Amato, J.F.R., Seixas, S.A. & Amato, S.B. (2007) A new species of *Temnocephala* Blanchard (Platyhelminthes, Temnocephalida) ectosymbiont on creeping water bugs, *Cryphocricos granulosus* De Carlo (Hemiptera, Naucoridae) from southern Brazil. *Revista Brasileira de Zoologia*, 24, 1043–1051.

Cannon, L.R.G. (1993) New temnocephalans (Platyhelminthes): ectosymbionts of freshwater crabs and shrimps. *Memoirs of the Queensland Museum*, 33, 17–40.

Cannon, L.R.G. & Sewell, K.B. (1995) Craspedellinae Baer, 1931 (Platyhelminthes: Temnocephalida) ectosymbionts from the branchial chamber of Australian crayfish (Crustacea: Parastacidae). *Memoirs of the Queensland Museum*, 38, 397–418.

Damborenea, M.C. (1992) Especies de *Temnocephala* (Platyhelminthes, Temnocephalidae) de crustáceos y moluscos de la Argentina. *Iheringia*, Série Zoologia (72), 3–21.

Damborenea, M.C. & Cannon, L.R.G. (2001) On neotropical *Temnocephala* (Platyhelminthes). *Journal of Natural History*, 35, 1103–1118.

Dioni, W.L. (1967) Temnocephalas Argentinas – III *T. pignalberiae* y *T. santafesina* novae species de *Trichodactylus* del Paraná médio. *Acta Zoológica Lilloana*, 23, 349–360.

Magalhães, C. (2003) Famílias Pseudothelphusidae e Trichodactylidae, pp. 143–287. *In*: G.A.S. de Melo (Ed.). Manual

de Identificação de Crustacea Decapoda de Água Doce do Brasil. São Paulo, Editora Loyola, 429pp.
Magalhães, C. (2005) Malacostraca – Eucarida, Brachyura Pseudothelphusidae and Trichodactylidae. Available online at: http://acd.ufrj.br/mndi/Carcinologia/hp/taxt/ Brachyura3.htm. [Accessed: 8 August 2005].
Magalhães, C. & Türkay, M. (1996) Taxonomy of the neotropical freshwater crab family Trichodactylidae. I. The generic system with description of some new genera (Crustacea: Decapoda: Brachyura). *Senckenbergiana biologica*, 75, 63–95.
Pignalberi, C.T. (1962) Breves noticias sobre la presencia de Temnocéfalos en cangrejos argentinos – Sessiones científicas Asoc. Cienc. nat. Litoral. *Anales del Museo F. Ameghino*, Santa Fe 1, 29–30.
Rego, A.A. (1982) Identificação de Temnocecephalida (Turbellaria) e de plerocercos de Trypanorhyncha (Cestoda) encontrados em caranguejos de água doce. *Revista Brasileira de Biologia*, 42, 275–278.
Seixas, S.A., Amato, J.F.R. & Amato, S.B. (2010) Redescription of *Temnocephala iheringi* (Platyhelminthes: Temnocephalida) based on specimens from *Pomacea canaliculata* (Mollusca: Ampullariidae) of the state of Rio Grande do Sul, Brazil: the possible type host and type locality. *Zoologia*, 27, 245–257.
Sewell, K.B. & Cannon, L.R.G. (1998) New temnocephalans from the branchial chamber of Australian *Euastacus* and *Cherax* crayfish hosts. *Proceedings of the Linnean Society of New South Wales*, 119, 21–36.
Sewell, K.B., Cannon L.R.G. & Blair, D. (2006) A review of *Temnohaswellia* and *Temnosewellia* (Platyhelminthes: Temnocephalida: Temnocephalidae), ectosymbionts from Australian crayfish *Euastacus* (Parastacidae). *Memoirs of the Queensland Museum*, 52, 199–280.